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U. S. DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE

RESEARCH PROGRESS REPORT

AGRICULTURAL RESEARCH, P.L. 85-934 and P.L. 89-106

4. FROM (Name and address of grantee)

Department of Soil and Crop Sciences
Texas Agricultural Experiment Station-TAMU

1. GRANT NO.
12-14-100-9894(75)

2. REPORT NO.
Final

3. PROJECT NO.

SE4-4-1(Gr)

5. REPORT PERIOD

(Should coincide with Fiscal Report requirements)

FROM

July 1, 1968

TO

June 30, 1972

6. PROJECT TITLE

Basic Studies on the Properties of Sunflower Important to Food Use.
Contract No U.S.D.A., A.R.S. 12-14-100-9894 (75)

7. SIGNIFICANT FINDINGS Sodium, potassium, zinc, iron, magnesium, copper, and calcium metals were quantified in six varieties of sunflower and related to discoloration due to metal-amino acid-quinone complexes. Relationship to pH was demonstrated. Specific gravity and airspace in seeds was found to not be related to ease of decortication. Sodium stearoyl-2-lactylate increases loaf volume of white bread enriched with 3% sunflower meal.

8. SUMMARY OF PROGRESS (Give concise summary of progress for this report period.) (If additional space is required, use ARS FORM 52A)

TERMINAL REPORT

Discoloration. The determinants responsible for the off-color problem were delineated. These determinants are: oxidation of the phenolic chromogens, chlorogenic and caffeic acid by metal catalysts and at an alkaline pH. The quinones formed by this oxidation bond with amino acids at pH 9.3 or greater and also form metal-amino acid-quinone complexes. Chlorogenic and caffeic acid will chelate with iron, copper, magnesium and calcium at an alkaline pH. Iron and copper will also chelate at an acid pH. The quinones formed produce a yellow color which turns amber upon further oxidation. The detrimental off-color results from the bonding, complexes and chelation of the above determinates.

The following minerals were quantified from six sunflower varieties: sodium, potassium, zinc, iron, magnesium, copper, and calcium.

Decortication. Twenty-five varieties of seeds were selected from the 1970 Regional Sunflower Yield Test. These varieties included birdseed, confectionary, and high oilseed types. The specific gravity and average airspace was computed for each sample. The airspace was considered to be an indication of how tightly the seed coat adhered to the kernel. The seeds were then decorticated in a modified waring blender and the percentage of complete, partial, and non-decorticated seeds was determined. After statistical analysis it was discovered that the difficulty of decortication was not related to specific gravity or to average airspace. However, a great varietal difference was found to exist in the degree of difficulty of decortication. Since this variation is not related to the physical characteristics; specific gravity and air space, it can be attributed to inherent differences in present breeding lines. Selection of seed stock should be made with these differences in mind.

Bread. Sunflower enriched bread at the levels of 1 1/2%, 3%, 5%, 17%, and 30% levels was baked. A dough conditioner, sodium stearoyl-2-lactylate, was added. In all of the samples the dough conditioner greatly improved the loaf volume. The 1 1/2% loaf was very similar to the wheat flour control. At the 3% level, the loaf volume was greater than that of the control wheat flour loaf. The color, texture, and flavor of the 3% loaf were equal to or greater than the control. An enrichment of greater than 17% produced an unacceptable loaf.

1018 (continued on ARS Form 52A)

9. SIGNATURE OF PRINCIPAL INVESTIGATOR IN CHARGE

E.G. Burns

M.H.B.

10. DIRECTOR OF RESEARCH INSTITUTION

W.O. Skunkel

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RESEARCH PROGRESS REPORT
AGRICULTURAL RESEARCH, P.L. 85-934 AND P.L. 89-106
(Continuation Sheet)

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PROJECT NO.	
12-14-100-9894(75)	
REPORT PERIOD	
FROM	TO
July 1, 1968	June 30, 1972

SUMMARY OF PROGRESS (Continued)

Contract No. U.S.D.A., A.R.S. 12-14-100-9894 (75)

Page 2.

Publications.

1. Burns, E. E., Talley, L. J., and Brummett, B. J. Sunflower Utilization in Human Foods. Accepted for publication. Cereal Science. Amer. Assoc. Cer. Chem. 1972 Copy attached.
2. Talley, L. J., Brummett, B. J., and Burns, E. E. Sunflower Food Products. MP-1026 Texas Agric. Expt. Sta., 1972. Copy attached.
3. Brummett, B. J. and Burns, E. E. Pigment and Chromogen Characteristics of Sunflower Seed, Helianthus annuus. Jour. Food Sci. 37(1) 1972. Copy attached.



SUNFLOWER UTILIZATION IN
HUMAN FOODS

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Texas A&M University

Analysis of physical and organoleptic properties indicate that dehulled sunflower kernels can be used in human food formulations. Whole kernels are acceptable as a nut substitute in bakery and confectionary products (Figure 1). Development of color and textural changes during roasting of whole kernels can be objectively measured and related to taste panel scores. Flavor and textural changes in products appear to be of minor importance.

High protein sunflower meal can be incorporated into new human food formulations. The kernels and meal can also be combined with readily available inexpensive staples to develop nutritious enriched products that are traditionally accepted by the populations of various countries.

Conventionally, the meal which is left after pressing or extracting oilseeds has been used for animal and bird feeds (1). Clandinin (2) and Robinson, et al. (3) have reported that the meal makes a high quality protein feed when incorporated in poultry and livestock feeds. This meal is approximately 50% protein and the cost of production is as low as 8 to 12 cents per pound of protein (4). As the inefficient process of converting plant protein to animal protein becomes more costly, even well developed countries will turn more and more to oilseed proteins to supplement nutritional needs of humans (5).

The increase in demand for edible oils, the increase in price for sunflower seeds, the decrease in availability of Soviet Union sunflower oil, and the decrease in the production of other edible oils has caused an increase in production of sunflower throughout the world (6).

In 1971 (7) approximately 85 dollars per ton was being given for sunflower meal as compared to approximately 110 dollars per ton for soybean meal. This difference is due to the strong demand for soybean meal for human food consumption as well as for livestock feed. The increasing knowledge of utilization of sunflower meal for human foods could rectify this difference in the near future.

It is conceivable that meal could satisfy a large portion of the current market for high protein products. Investigations indicate that sunflower can be utilized very successfully in human food formulations.

Several types of sunflower products are now available. Whole sunflower seeds are used primarily for oil production and the bird-seed trade (8). Both hulled and dehulled kernels are found in the snack and health food trades.

Sunflower Meal

The removal of oil from dehulled sunflower seeds produces a high protein meal that is quite acceptable for incorporation into products with a normal pH range of 5 to 7. The work of Talley et al. (9) has shown that the meal has excellent stability and exhibits an attractive cream color and relatively bland nutty flavor under various conditions of time, temperature, and humidity.



As early as 1947, the University of Illinois Home Economics Department (10) concluded that the meal was a rich source of highly digestable and nutritive protein, calcium, and B vitamins for human foods. High protein chips and bread made from the meal are acceptable when processing conditions do not induce oxidation of the chlorogenic acid.

A major problem in using whole seeds in human foods is the tendency of both raw and roasted kernels to develop rancidity unless held under cold storage. The major oxygenated fatty acids in sunflower seed oil that could affect flavor stability and nutritional value have been isolated and identified by workers at the Northern Regional Research Laboratory in Peoria, Illinois (11).

Chromogenesis

A major problem in using sunflower meal in human foods is the presence of hulls and chlorogenic acid as pointed out by Pomenta (12). Both of these components cause undesirable discoloration of the meal under certain conditions. Hulls can also cause excessive bulk and fiber. Problems with hulls were also experienced by the University of Illinois Home Economics Department when comparing the composition of sunflower flour with other flours and flour supplements. The sunflower was incorporated into many baked products, but the presence of hulls in the meal produced some undesirable discoloration (Table 1).

The pH value has a major effect upon the chromogenesis of chlorogenic acid. At various pH levels, the meal turns from white, pH 3; to beige, pH 6; to green, pH 9; to brown, pH 12. The white color was apparently due to precipitaiton of water soluble proteins

in a highly acid solution while green and darkening was due to oxidation of chlorogenic acid.

Chemical and physical analysis of defatted sunflower meal indicate discoloration to be mainly due to the oxidation of chlorogenic acid at alkaline pH levels. This off-color was originally considered to be caused by a substance called helianthotannic acid (13). Later investigation by Gorter (14) identified this compound as chlorogenic acid. Investigations by Milic et al. (15), Pomenta (12), Mikolajczak et al. (16), and Brummett and Burns (17) have disclosed the following phenolic compounds in sunflower seeds: chlorogenic, caffeic and quinic acid. The oxidation of one or more of these phenols, whether by oxygen at an alkaline pH or enzymatically by polyphenol oxidase, produces an off-color (17, 18).

Protein

The isolation of protein from sunflower meal has been investigated by numerous authors. Osborne and Cambell (13), Smith and Johnsen (19), Joubert (20), Sosulski and Bakal (21), Gheyasuddin et al. (22), and Gheyasuddin et al (23). These authors are in agreement that the isolate has a high nutritional value; however, they also found that under conventional alkaline extraction or acid precipitation the isolate possessed a detrimental off-color.

Osborne and Cambell (13) and Smith and Johnsen (19) had little success in their attempts to extract chlorogenic acid from the meal. Joubert (20) was able to completely extract chlorogenic acid from the meal. However, all of these extractions denatured the protein and are hardly feasible for industrial use. Perhaps the work by

Gheyasuddin et al. (22) can be developed into a profitable extraction process for the industry. They used a reducing agent, sodium sulfite, during an alkaline extraction, and acid precipitation, and an alcohol wash to obtain a white protein isolate.

The use of salt to extract sunflower protein was done by Smith and Johnsen (19) and Gheyasuddin et al. (23). Smith and Johnsen (19) reported a brown colored isolate instead of the dark green color imparted from an alkali extraction. Gheyasuddin et al. (23) reported a slight increase in total nitrogen extracted with both sodium chloride and calcium chloride. Sosulski and Bagal (21) and Gheyasuddin et al. (23) are in close agreement concerning their reported values for sunflower protein fractions. They both used Osborne's solubility classification and obtained the following mean values: water soluble - albumins, 20%; salt soluble - globulins, 56%; alcohol soluble - prolamins, 3%; dilute alkali - glutelins, 15%; and residue, 6%.

Sunflower protein can be a very valuable source of human foods in addition to the high quality oil. The National Academy of Sciences (24) published the average composition of selected oilseed meals and reported that sunflower has a higher protein content than either cottonseed or soybean (Table 2).

Amino Acids

One major item to consider is the nutritional quality of the sunflower protein or the amount of nitrogen available as amino acids. Hydrolyzing the protein with HCl resembles digestion in the body. The nitrogen available as amino acids was determined by Bandemer and Evans (25) with ion exchange chromatography. Whole hen's eggs were included as representative of animal protein of high nutritive value.

As can be seen in Table 3, sunflower seed has the highest percent of protein of any plant material tested. Earle et al. (26) also compared sunflower protein to whole hen's egg and reported a high value, 89 as compared to 100 for whole hen's egg. And when calculating the percent nitrogen available as amino acid from Table 3, the 53.4% of the fat extracted sunflower meal outstrips the 39.2% of the fat extracted meal.

The amino acid composition of sunflower protein can be seen in Table 4 (26). Although the value of the amino acid composition is below that of some animal proteins, it is quite similar to that found in other plant seeds. Lysine and Isoleucine are the limiting essential amino acids for human nutrition.

Enriched Bread

Product work by Talley et al. (9) at Texas A&M University involved white bread. Bread was baked substituting 3%, 17%, and 30% of the wheat flour with defatted sunflower meal ground in a Willey Mill to pass through a 40 mesh screen. Immediately upon removal from the oven, bread volume was measured in a National Loaf Volume Meter. The bread was then cooled 2 hours before cutting to grade for the quality factors of appearance and symmetry, break and shred, crust color, texture grain, and crumb color. The protein content of the bread was determined using the A.O.A.C. (1965) method for bread and bread products. The percent nitrogen was determined by the Kjeldahl Method, and multiplied by 5.30 to calculate protein content. Addition of sunflower meal substantially increased the bread protein. The wheat flour used in making the bread had 8.6% protein while the

sunflower meal had 46.8% protein. The 0%, 3%, 17%, and 30% enriched bread formulations had 11.6%, 13.4%, 19.0%, and 23.6% protein, respectively. The 17% and 30% formulas produced a very compact and heavy loaf that would be undesirable for bread (Figure 2). The 3% level of enrichment, however, produced an attractive loaf with a pleasant and distinctive nutty flavor.

Chemical and Physical Studies of Sunflower Types

The chemical and physical characteristics of selected types of sunflower types have been studied (12). These types were selected as representative of the commercial seeds grown in various parts of the U.S.A. and were supplied by Dr. Kinman of the U.S.D.A. (Table 5). The crude protein of these seeds ranged from 23.85% to 33.60% indicating that all could be used economically as a source of protein. Histochemical studies of the sunflower seed tissues revealed that the main location of chlorogenic acid is the aleurone or protein containing bodies of the cell.

This close association makes solvent extraction of the chlorogenic acid rather difficult. However, the chlorogenic acid content of freshly harvested seeds decreases upon storage at 5°C., 15°C., and 40°C. according to Pomenta and Burns (27). Work by Gheyasuddin and associates (22) has resulted in a colorless protein isolate. Work by Brummett and Burns (17) has revealed sunflower meal to be a rich source of vitamin A.

Acknowledgments

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TABLE 1.

Sunflower Seed Flour: Composition Compared With That
of Other Flours and Flour Supplements

Food	Mois-ture	Food Energy Per 100 grams	Crude Protein	Fat	Carbo-hydrate	Cal-cium	Phos-phorus	Vitamins per 100 grams			
								Vitamin A	Thiamine	Ribo-flavin	Niacin
Sunflower seed flour*	4.6	362	52.8	4.5	27.5	.57	.58	1	3.6	.48	30.0
Buckwheat flour, light	12.0	354	6.3	1.1	79.7	.01	.09	0	.31	.08	2.1
Cornmeal white, degerminated	12.0	355	7.5	1.1	78.8	.01	.14	0	.16	.09	.9
Rye flour, light	11.0	358	8.9	.9	78.5	.02	.28	0	.15	.07	.9
Soy flour*	9.0	283	42.5	6.5	13.6	.24	.61	110	.82	.34	2.6
Wheat flour, patent	12.0	355	10.8	.9	75.9	.02	.09	0	.07	.03	.8
Wheat flour, patent, enriched	12.0	355	10.8	.9	75.9	.02	.09	0	.44	.26	3.5
Wheat flour, whole	11.0	360	13.0	2.0	72.4	.04	.38	0	.56	.12	5.6
Milk, dried defatted	3.5	359	35.6	1.0	52.0	1.30	1.03	40	.35	1.96	1.1

*Defatted

Source: Illinois Experiment Station Bulletin, 1947, Number 608.

TABLE 2.

Average Composition of
Selected Oilseed Meals
(Solvent Extracted)

	Sunflower Meal	Cottonseed Meal	Soybean Meal
Moisture	7.0	9.0	11.0
Ash	7.7	6.5	5.8
Crude Fiber	11.0	11.0	6.0
Ether Extract	2.9	1.6	0.9
Protein (N X 6.25)	46.8	41.6	45.8

Source: National Academy of Sciences, 1964. National Research Council, Publication 1232.

TABLE 3.

Distribution of Nitrogen
in Certain Hydrolyzates
(Hydrolized in 20% HCl)

Material	Protein, % (N X 6.25)	Amino Acid N	N as % Total N	Ammonia
Wheat	9.9	67.7	13.7	
Corn	10.8	74.6	16.5	
Rice	6.6	75.0	14.0	
Barley	9.6	67.8	11.5	
Millet	14.0	72.4	17.2	
Navy Beans	24.0	78.1	10.9	
Sunflower Seed*	66.2	80.6	12.3	
Soybean*	49.8	78.7	10.6	
Whole Egg	72.8	86.4	8.5	

*Defatted

Source: Bandemer, S. L. and Evans, R. J. 1963. The Amino Acid Composition of Some Seeds.
Journal of Agricultural and Food Chemistry 11, 134.

TABLE 4.

Amino Acid Composition of Meal Prepared From
Defatted Sunflower Kernels (g/16g nitrogen)

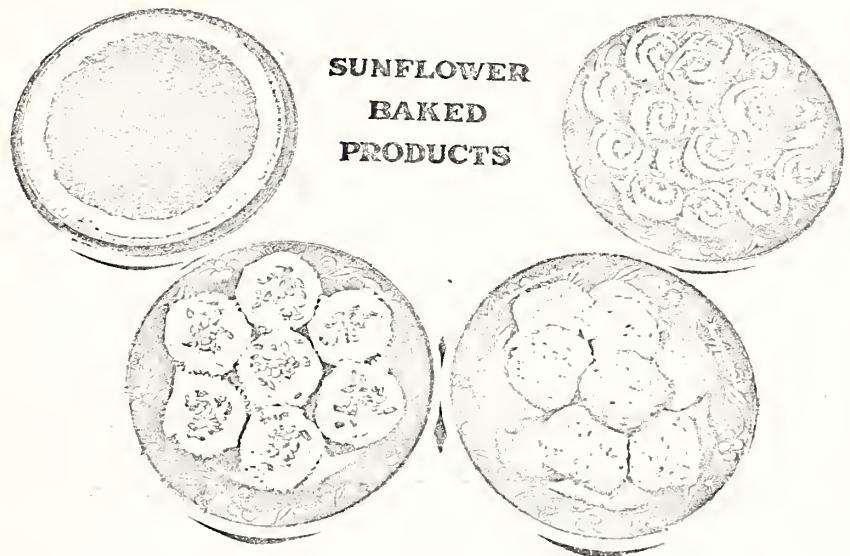
Amino Acid	Mean for Seven Varieties	Range	Hull from Semena Variety
Lysine	3.77	3.4 - 4.2	5.5
Methionine	1.91	1.7 - 2.1	1.6
Cystine	1.82	1.6 - 2.2	---
Phenylalanine	4.70	4.6 - 4.8	4.3
Tyrosine	2.65	2.6 - 2.8	2.3
Tryptophan	1.11	1.0 - 1.2	---
Isoleucine	3.97	3.9 - 4.1	3.7
Leucine	6.13	6.0 - 6.2	6.1
Threonine	3.18	3.0 - 3.4	3.9
Valine	4.76	4.3 - 5.1	4.7
Histidine	2.47	2.4 - 2.6	2.9
Arginine	8.91	8.4 - 9.2	5.7
Glycine	5.05	4.8 - 5.3	6.8
Serine	3.89	3.4 - 4.1	4.7
Alanine	4.07	3.9 - 4.3	4.6
Aspartic Acid	8.70	8.4 - 8.9	9.5
Glutamic Acid	20.95	19.6 - 21.7	13.3
Proline	5.01	4.5 - 5.3	4.5
Ammonia	2.18	1.8 - 2.7	2.4
Total	95.23		86.5

Source: Earle, F.R., Vanetten, C.H., Clark, T. F., and Wolff, I.A., 1968.
Compositional Data on Sunflower Seed. Journal of the American
Oil Chemists' Society, 45,876.

TABLE 5.
Chemical Composition of Selected
Raw Sunflower Kernels

Variety	% Lipids	% Crude Protein	% Total Sugars	Ash	% Crude Fiber
Mingren	45.66	30.66	4.91	4.44	12.19
Arrowhead	45.13	33.60	4.69	4.65	9.73
Greystripe	45.37	30.71	6.28	4.63	10.04
Manchurian	44.64	30.98	5.94	3.81	11.28
Krasnodarets	49.59	28.86	4.41	4.22	10.97
Peregovik	50.74	27.18	4.99	4.21	10.65
Valley	52.32	27.11	4.26	4.40	9.98
T 56002	48.12	31.32	4.91	4.10	9.35
P--21 ms X HA 60	52.66	23.85	4.98	4.03	12.47
VNIIMK	49.73	28.82	5.25	4.39	9.62

Source: Pomenta, J. V. 1970. Chemical and Physical Characteristics of Selected Types of Sunflower Seeds. M. S. Thesis, Texas A&M University, College Station, Texas.



SUNFLOWER
BAKED
PRODUCTS

Figure 1. Sunflower Baked Products

SUNFLOWER ENRICHED BREAD

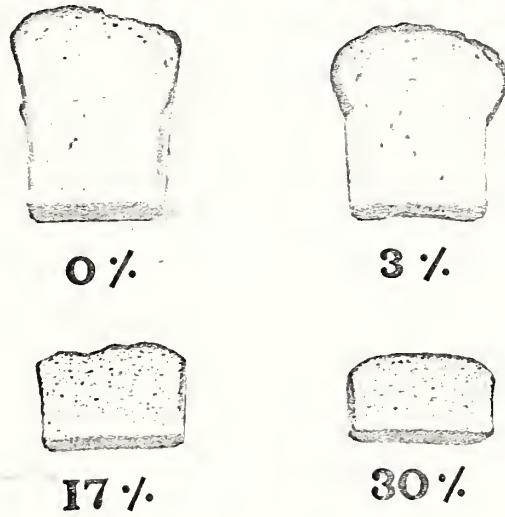


Figure 2. Sunflower Enriched Bread

PIGMENT AND CHROMOGEN CHARACTERISTICS OF SUNFLOWER SEED, *Helianthus annuus*

INTRODUCTION

THE EDIBLE OIL from sunflower seeds has achieved worldwide acceptance. The meal from which this oil is extracted has been utilized principally as animal feed but has potential as human food. Undesirable changes in color of the meal can occur during and after further processing. Delineation of the presence of naturally occurring pigments or chromogens in the various types of sunflowers is fundamental to the utilization of this crop for human food.

EXPERIMENTAL

Sample material

The names and colors of the selected types of sunflower seeds used for analysis are presented in Table 1. Since this study involved the analysis of the pigments and chromogens in the sunflower kernel and hull, six cultivars were selected with a range in seed coat color from white to striped, to essentially black. For the most part these are representative of the commercial types grown in the USA, and were supplied by the Oilseed and Industrial Crop Section, Dept. of Soil & Crop Sciences, Texas A&M University, in cooperation with the Oilseed & Industrial Crops Research Branch, Plant Sciences Research Div., ARS, USDA. The seeds were grown in College Station in 1970 and were part of the Regional Sunflower Yield Test. Environmental effects on composition were thus minimized. Relative values should not vary

extremely. The seeds were randomly drawn from replicate samples and stored in closed containers at 5°C. The color descriptions were as reported by Pomenta (1970), in addition to the Kitale type which is white.

Surface color of the seeds

The Gardner Automatic Color Difference Meter was utilized to determine the surface colors of the kernels and hulls. Since the color was not evenly distributed, especially on the hull, four replicates were measured. The results are reported in Gardner color values of "L," "a" and "b."

Kernels and hulls

The seed weights were obtained by averaging the weight of 10 samples of 10 seeds. These samples were then decorticated by hand to obtain kernel and hull weights. The decortication of the individual seeds by hand was necessary because of undeveloped kernels within some of the seeds.

Lipid content

Kernels and hulls were thoroughly dried in a forced draft oven and the lipid content measured in a Newport Nuclear Magnetic Resonance Analyzer, Magnet Type 10.

Sugar content

The method of Dubois et al. (1956) was used to determine total sugars. For the determination of reducing sugars, the Talbert and Smith (1959) dinitrophenol method was utilized. Glucose standards were used to construct standard curves. The difference between the two percentages was considered to be non-reducing sugars.

β-carotene content

Extraction of β-carotene was according to the methods of Zscheile and Porter (1947) and from the *Official Methods of Analysis*, AOAC (1965). Concentrations were calculated according to the AOAC formula based on Beer's law, and reported as mg/100g.

The mixture of acetone and hexane, 30% and 70% respectively, from the AOAC (1965) method was found to be the best solvent. The blending, filtering, and separatory funnel techniques of Zscheile and Porter (1947) gave a rapid and accurate extraction. All work was carried out in reduced light and read in a Beckman Model DB spectrophotometer and recorded with a Beckman potentiometric recorder. Variation was found when samples were allowed to stand in amber flasks and in a 0°C room overnight.

Isolation of the chromogens

The use of petroleum ether and warm aqueous ethanol to extract chromogens from seeds has been reported by numerous authors (Rao et al., 1939; Farooq et al., 1953; Laumas and Seshadri, 1958). Therefore, the sunflower kernels were extracted with petroleum ether for 24 hr in a Soxhlet extractor, and then an additional 24 hr in 90% alcohol. An identical type extraction was utilized for the extraction of the chromogens from the sunflower hull. 400g samples were used for both the kernels and the hulls.

The alcohol extracts were reduced to suitable volumes in a rotary-vacuum evaporator and saturated lead acetate was added in sufficient quantity to precipitate the chromogen compounds. Next, dilute sulfuric acid was added to

Table 1—Names and colors of sunflower seed samples

Names	Colors
Kitale	White
Greystripe	White with narrow dark grey stripes
Mingren	Grey and white stripes
Arrowhead	Dark grey and white stripes
P-21 ms × HA60	Black with narrow white stripes
Peredovik	Black and dark grey stripes

Table 2—Gardner color values for selected types of sunflower seeds^a

Cultivars	Kernel			Hull		
	L	a	b	L	a	b
Kitale	22.60a	2.35a	17.80ac	61.60a	5.73a	12.65a
Greystripe	24.23b	1.88b	18.65b	48.15b	4.63b	12.50a
Mingren	22.58a	2.13c	17.65c	37.68c	3.05c	10.08b
Arrowhead	22.35a	2.58d	19.15d	30.63d	1.93d	8.75c
P-21 ms × HA60	22.18a	2.40ad	18.23ab	20.03e	.28e	4.70d
Peredovik	22.58a	2.28ac	18.30ab	18.95e	.10e	4.00e

^aMeans followed by the same letter are not significantly different at the 5% level of probability of the Duncan Multiple Range Test.

Table 3—Lipid content of selected types of sunflower seeds^{a,b}

Cultivars	Kernel %	Hull %
Kitale	50.6a	7.8a
Grey stripe	45.5b	5.7b
Mingren	48.3c	6.2c
Arrowhead	45.7b	5.3b
P-21 ms × HA60	50.0a	8.6d
Peredovik	49.6a	10.9e

^aDry weight basis^bMeans followed by the same letter are not significantly different at the 5% level of probability of the Duncan Multiple Range Test.

The combination of paper chromatography with spectrophotometric methods enables identification of flavonoid compounds (Robinson, 1963). Spectra were determined at room temperature with a Beckman Model DB Spectrophotometer and recorded with a Beckman potentiometric recorder.

Quantitative measurement of the chromogens

Light transmittance characteristics of the purified chromogens and authentic compounds were measured using the Beckman Model DB Spectrophotometer. Standard reference curves were constructed. The pigments from each of the selected types of sunflower seed extracts were measured and calibrated with their appropriate standard curves to determine concentration. The procedure of Pomenta and Burns (1971) was used for the determination of chlorogenic, caffeic and quinic acids.

free the chromogen compounds from the lead precipitate. This was done by a series of washings and centrifugations of the lead precipitate.

The separation of the chromogen compounds from the previously isolated extract was performed by streaking 2–4 ml of the extract in a narrow band upon Whatman No. 3MM paper. The chromatograms were developed by using descending chromatography in an equilibrated atmosphere. The solvent used for the first development was n-butanol-acetic acid-water mixture in a 6:1:2 ratio. After development and drying, the individual bands were located by means of an ultraviolet light, by spraying, or by fuming with a suitable reagent. The located bands were then cut out, eluted with 90% aqueous alcohol and the eluate concentrated for further purification. These steps were repeated with these solvent systems in the following sequence: n-butanol-acetic acid-water, 6:1:2; n-butanol-water, saturated; acetic acid-water, 10:90 and 50:50; m-cresol-acetic acid-water, 50:2:48; ethyl acetate-water, saturated; benzene-pyridine-water, 100:1:100; and chloroform-ethyl alcohol-water, 8:2:1 until solutions were obtained which were chromatographically pure.

Characterization of the chromogens

Characterization through the use of paper chromatography is dependent upon the multiple determination of the two physical characteristics: mobility on paper as indicated by *R_f* value, and color development. *R_f* values and colors were helpful in the identification of chromogens. Authentic chromogens were used as reference compounds to confirm accuracy.

RESULTS & DISCUSSION

Surface color of the seeds

As previously mentioned, the six cultivars selected for this study ranged in seed coat color from white, to striped, to black (Table 1). They were selected to establish correlations between kernel color, seed coat color and chromogen content. Negative correlation coefficients of -0.56 (*P* < .05) and -0.55 (*P* < .05) were found to exist between the Gardner color value "b," degree of yellowness, and chlorogenic acid in the kernel and hull, respectively. A negative correlation coefficient of -0.69 (*P* < .01) was found between the Gardner color value "b" and the percent lipids in both the kernel and the hull. A stepwise decrease in the Gardner color value "L," lightness, was found to exist among five of the six cultivars selected for this study. This was to be expected since the seeds ranged from light to dark. The two dark seeds, P-21 ms × HA60 and Peredovik, were not significantly different. The Gardner color values for the selected types of sunflower seeds can be seen in Table 2.

Lipids

The percent lipid content of the kernels and hulls can be seen in Table 3. The levels for the kernel ranged from

45.5–50.6%. This is comparable to that reported by Kinman and Earle (1964), Kinman (1966), Pomenta (1970) and Ravagnan (1970). The lipid content of the hulls ranged from 5.3–10.9%. This could prove to have a dual interest, not only to the livestock feeder, but possibly to the edible oil processors as well. As previously mentioned, a significant correlation coefficient existed between the percent lipids and the surface color of both the kernel and the hull. Significant correlation coefficients were found between percent lipids and nonreducing sugars in both the kernel and the hull, respectively [0.68 (*P* < .01) and 0.56 (*P* < .05)].

Sugars

The percent sugars, both reducing and nonreducing, can be observed in Table 4. Positive correlation coefficients, 0.93 (*P* < .001) and 0.56 (*P* < .05), were obtained between reducing sugar content and chlorogenic acid content in both the kernel and the hull, respectively. This is in agreement with Pomenta and Burns (1971), and should prove to be important to the plant breeder. An analysis for reducing sugars can be carried out faster and simpler than the more complex and lengthy chlorogenic acid analysis.

The total sugar content of the hulls might interest the livestock feeder as an economical source of energy.

Pigments

Preliminary spectrophotometric determinations indicated the possible presence of kaempferol, a flavonoid chromogenic compound. However, further work showed that a mixture of chlorogenic, caffeic, and quinic acids exhibited an ultra-violet spectra nearly identical to that of kaempferol.

Flavonoid pigments were not found in either the kernel or the hull. However, β-carotene was present in both. The content of β-carotene for the six cultivars in this study can be seen in Table 5. Appreciable amounts were found. A range of 0.398–1.096 mg/100g was established for the kernels and 0.056–0.194

Table 4—Sugar content of selected types of sunflower seeds^{a,b}

Cultivars	Kernel		Hull	
	Reducing %	Nonreducing %	Reducing %	Nonreducing %
Kitale	.81a	1.015a	.23a	.061a
Grey stripe	.88b	.729b	.31b	.083b
Mingren	1.15c	.662c	.36c	.096c
Arrowhead	.88b	.704d	.35cd	.094cd
P-21 ms × HA60	.85ab	1.013a	.32bd	.085bd
Peredovik	.96d	.751e	.53e	.142e

^aDry weight basis^bMeans followed by the same letter are not significantly different based on the 5% level of probability by the Duncan Multiple Range Test.**Table 5—Beta carotene content^{a,b}**

Sunflower cultivar	mg/100g	
	Kernel	Hull
Kitale	.398a	.143a
Grey stripe	1.096b	.194b
Mingren	.550c	.056c
Arrowhead	.535c	.105d
P-21 ms × HA60	.494ac	.181b
Peredovik	1.024b	.194b

^aDry weight basis^bMeans followed by the same letter are not significantly different based on the 5% level of probability by the Duncan Multiple Range Test.

Table 6-Chromogen content of selected types of sunflower seeds^{a,b}

Cultivars	Kernel			Hull		
	Chlorogenic acid %	Caffeic acid %	Quinic acid %	Chlorogenic acid %	Caffeic acid %	Quinic acid %
Kitale	.541a	.194ac	.126a	.312ab	.139ab	.124a
Greystripe	.463a	.177a	.169a	.410a	.171ac	.131a
Mingren	.831b	.286b	.131a	.319ab	.137ab	.094a
Arrowhead	.531a	.232abc	.146a	.319ab	.182c	.092a
P-21 ms x HA60	.569a	.226abc	.155a	.297ab	.103abd	.123a
Peredovik	.851b	.250bc	.255b	.254b	.088d	.100a

^aDry weight basis^bMeans followed by the same letter are not significantly different at the 5% level of probability of the Duncan Multiple Range Test.

mg/100g for the hulls. The range in other food products has been reported as: carrots 0.020–13.600 mg/100g; sweet potatoes 0.200–3.940 mg/100g; tomatoes 0.100–1.400 mg/100g; maize 0.100–1.100 mg/100g; wheat 0.108–0.380 mg/100g and soybeans 0.018–0.970 mg/100g (Goodwin, 1954). This information is of particular value regarding the utilization of sunflower meal.

The single documented report of β -carotene in sunflowers seems to be Illinois Experiment Station Bulletin, No. 608, published in 1947. One International unit of Vitamin A/100g, or 0.00006 mg/100g of β -carotene, was reported in sunflower seed flour. This amount is extremely low when compared to the high of 1.096 mg/100g found in the material used in the present study (Table 5).

Interesting correlation coefficients were found to exist among quinic acid content and β -carotene content in both the kernel, 0.91 ($P < .001$) and the hull, 0.83 ($P < .001$). This may suggest the influence of one of these molecules on the synthesis of the other.

Chromogens

Chlorogenic, caffeic and quinic acids were found in both the kernel and the hulls. The quantities of the three acids are

presented in Table 6. These quantities, for the kernel, are in agreement with similar work done by Milic et al. (1968), Pomenta (1970) and Mikolajczak et al. (1970).

When the percent of these acids or phenols for the whole seed are taken into consideration, the content in the kernel is higher than in the hulls for each of the cultivars analyzed. This eliminates the hypothesis that hull fractions left in meal are the major contributors to the phenolic off-color problem.

β -carotene, chlorogenic acid, caffeic acid, and quinic acid were the only pigments and chromogens found. This lends support to the theories of E.E. Burns (Personal communication, 1969) and Gheyasuddin et al. (1970) that the off-color is due to the oxidation of chlorogenic acid. This oxidation commonly occurs when the meal undergoes an alkaline pH change during processing into human food products. The discoloration can also occur enzymatically by the action of polyphenol oxidase (Sondheimer, 1964).

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